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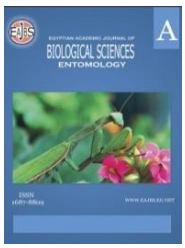

Cytopathological Impacts of Certain Plant Growth Regulators on The Circulating Hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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ABSTRACT

The greater wax moth *Galleria mellonella* is a worldwide insect pest damaging wax combs and feeding on bee-hive products. The objective of the current study was to investigate the cytopathological impacts of four plant growth regulators (PGRs), *viz*., indole-3-acetic acid, indole-3-butyric acid, 2,4-Dichlorophenoxy acetic acid and 6-benzyladenine, on the circulating hemocytes of last (7^{th}) instar larvae of *G. mellonella*. For this purpose, the 3^{rd} instar larvae were force-fed on diet supplemented with LC_{50} values of these PGRs (0.24, 0.022, 0.16 & 0.085 ppm, respectively). These larvae were continuously fed on the treated diet throughout the larval stage. The important results could be summarized as follows. Five main types of the freely circulating hemocytes in the haemolymph of larvae had been identified as Prohemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytoids (OEs). Different qualitative disorders of the profile of each circulating hemocyte type were demonstrated in thelast instar larvae, such as destruction of cell nuclei and/or membrane, some extruded cytoplasmic contents, and production of some vacuoles in the cytoplasm. However, some of the tested PGRs failed to exhibit cytopathological impacts on certain hemocytes, since IAA and 6-BA failed to affect PLs, IBA and 6-BA failed to affect GRs, IBA and 6-BA failed to affect SPs and IBA and 6-BA failed to affect OEs. Therefore, IAA and 2,4-D may be recommended to use in the IPM program against *G. mellonella*

INTRODUCTION

The greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) is widely distributed throughout the world. Although the adults do not feed, because they have atrophied mouth parts, the voracious nature of larval feeding and tunneling usually leads to the destruction of the honeycomb, and subsequently to the death of weak colonies (Chandel *et al*., 2003; Awasthi and Sharma, 2013; Mohamed *et al*., 2014; Kwadha *et al*., 2017; Abo Elsoud *et al.*, 2021a, b; Altuntaş *et al*., 2021).

Because the majority of physical and biological measures seemed to be ineffective for satisfactory control of different stages of *G. mellonella* (Fraser, 1997; Coskun *et al*., 2006), the apiculture industry has traditionally relied on synthetic insecticides for controlling the serious insect pests in different parts of the world (Rehman *et al*., 2009; Ilyas *et al*., 2017) including *G. Mellonella* (Durmuş and Büyükgüzel, 2008; Sak and Uckan, 2009).These chemicals seemed to be ineffective against eggs of *G.*

mellonella (Owayss and Abd-Elgayed, 2007). Also, synthetic insecticides have adverse effects on non-target organisms and on the environment in general. In addition, they gradually accumulate in food materials and ultimately cause severe disease in human consumers (Owain *et al*., 2008; Ambethger, 2009; Łozowicka *et al*., 2012; Mahdi and Mohammed, 2017). Moreover, one of the serious problems that emerged with synthetic insecticide applications is the development of insect resistance toward the used insecticides (Yarahmadi *et al*., 2009; Sparks and Nauen, 2015; Maazoun *et al*., 2017). The *G. mellonella* has developed high resistance to many insecticides (Said *et al*., 2019). Besides these hazards, pesticides lead to oxidative stress, inhibition of physiological and biochemical pathways, induce toxicity, impede photosynthesis and negatively affect the yield of crops (Jan *et al*., 2020).

Natural compounds of plant origin may be efficient alternatives to conventional insecticides against *G. mellonella* (Abbasipour *et al*., 2009; Mahmoudvand *et al*., 2011; Basedow *et al*., 2012; Elbehery *et al*., 2016; Er *et al*., 2017) because they are relatively safe and have minimal residual effects on humans and the environment (Koul*et al*., 2008; Pavela, 2009). In different parts of the world, several researchers have focused on the effects of various plant growth regulators (PGRs) on different insect pests because these compounds may have considerable impacts on the survival, development and reproductive potential, as well as on other physiological processes and induction of the oxidative stress of many insect pests (Tsagkarakis *et al*., 2012; Prado and Frank, 2013; Abdellaoui *et al*., 2015; Kaur *et al*., 2016; Becerikli Aksan *et al*., 2022; Nagaratna *et al*., 2022).

Haematological studies are very important in insect physiology because the hemocytes perform various physiological functions in the body. The primary functions of hemocytes are: coagulation to prevent loss of blood, phagocytosis, encapsulation of foreign bodies in the insect body cavity, nodule formation, detoxification of metabolites and biologically active materials and distribution of nutritive materials to various tissues and stored them (for detail, see: Ling and Yu, 2006; Ribeiro and Brehelin, 2006; Siddiqui and Al-Khalifa, 2012a; Chavan *et al*., 2017; Ghoneim, 2019; Ghoneim *et al*., 2021). Therefore, the objective of the current study was to investigate the cytopathological impacts of four PGRs, *viz*., indole-3-acetic acid, indole-3-butyric acid, 2,4-Dichlorophenoxy acetic acid and 6-benzyladenine, on the profiles of circulating hemocyte types of the last instar larvae of *G. mellonella*.

MATERIALS AND METHODS

1. The Experimental Insect:

A culture of a susceptible strain of the greater wax worm *Galleria mellonella* L. (Lepidoptera: Pyralidae) was established in the Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt, and maintained for several successive generations under controlled conditions (27±2^{o}C, 65±5% R.H., photoperiod 14 h L and 10 h D). This culture was originally initiated by a sample of larvae kindly obtained from Desert Research Center, Cairo, Egypt. Larvae were transferred into glass containers, tightly covered with muslin cloth. Different techniques for preparing the artificial diet had been described by some authors (Metwally *et al*., 2012; Nitin *et al*., 2012). In the present culture of *G. mellonella*, an artificial diet was formulated depending on the method of Bhatnagar and Bareth, **(**2004). The diet contained maize flour (400 g), wheat flour, wheat bran and milk powder, 200 g of each. Also, it was provided with glycerol (400g), bee honey (400g), and yeast (100g). However, improved manipulation of different developmental stages had been done according to Ghoneim *et al*. (2019).

2. Plant Growth Regulators (PGRs):

Four PGRs were selected to be tested against *G. mellonella* in the present study, *viz*. Indole-3-Acetic Acid (IAA), a synthetic auxin compound with molecular formula: $C_{10}H_9NO_2$; Indole-3-butyric acid (IBA), a synthetic auxin compound with molecular formula: $C_{12}H_{13}NO_2$; 2,4-Dichlorophenoxy acetic acid (2,4-D), a synthetic auxin compound with molecular formula: $C_8H_6Cl_2O_3$ and 6-Benzyladenine (6-BA, or 6-Benzylaminopurine, BAP), a synthetic cytokinin compound with molecular formula: $C_{12}H_{11}N_5$. These PGRs were purchased from Millipore Sigma, Burlington, MA 01803, USA Merk Ltd., Cairo, Egypt.

3. Larval Treatment:

Depending on a toxicity bioassay using 100.0, 10.0, 1.0, 0.1, 0.01 and 0.001 ppm, LC_{50} values were calculated as 0.24, 0.022, 0.16 and 0.085 ppm for IAA, IBA, 2,4-D and 6-BA, respectively.For the evaluation of the disruptive effects of these PGRs on the profiles of different hemocytes, the 3rd instar larvae of *G. mellonella* were force-fed on anartificial diet supplemented with LC_{50}values of these PGRs. These larvae were continuously fed on the treated diet throughout the larval stage. The control larvae were fed on an untreated artificial diet. Haemolymph of the successfully moulted last (7th) instar larvae (treated or control) was subjected to examine the possibly affected morphology and structure of the main circulating hemocytes.

4. Collection of Haemolymphs:

The haemolymph samples were collected from the treated and control 7th instar larvae. Each haemolymph sample was obtained by amputation of one or two prothoracic legs, from coxa of the larva using fine scissors. Gentle pressure was done on the thorax for obtaining haemolymph drops by a non-heparinized capillary tube. Seven replicates were used and the haemolymph from two individuals was never mixed.

5. Hemocyte Identification and Influenced Haematological Parameters:

5.1. Hemocyte Identification:

Depending on the cell morphology, cytoplasmic ratio, cytoplasmic inclusions, shape of the nucleus and dye-staining properties, five main types of the freely circulating hemocytes in the haemolymph of last instar larvae of *G. mellonella* had been identified and distinguished based on the technique described by some researchers (Altuntaş *et al*., 2012; Blanco, 2016): Prohemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytoids (OEs).

5.2. Cytopathological Characterization:

The present hematological investigation was carried out only in the haemolymph of the last (7th) instar larvae after the feeding of the larval stage, starting from the 3rd instar larvae, on an LC_{50}-PGRs-treated artificial diet. The available haemocyte deformities were recorded. Photomicrographs of these deformities were prepared using a light microscope provided with a camera at a magnification of 10 X 40 = 400

6. Statistical Analysis of Data:

Data obtained were analyzed by the student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of the difference between means using GraphPadInStat© v. 3.01 (1998).

RESULTS

1. Identification and Description of Normal Circulating Hemocytes in Larvae of *G. mellonella*:

In the present study, the freely circulating hemocytes in haemolymph of the last (7th) instar larvae of *G. Mellonella* were identified into five main types, *viz*., Prohemocytes,

Plasmatocytes, Granulocytes, Spherulocytes and Oenocytoids, depending on the cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus. The important distinguishable characters of each type could be summarized herein.

1.1. Prohemocytes (PRs): ovoid cells, variable in size (3-7 µm wide and 6-8 µm long), with a large centrally located nucleus and a prominent nucleolus. Deeply stained cytoplasm was abundant and contained few organelles. In a few cases, some vesiculation of the plasma membrane was evidently observed (see Fig. 1).

1.2. Plasmatocytes(PLs):spindle-shaped cells (about 16x 4 µm) with alarge nucleus (40-50% of the cell volume). The nucleus was elongated, round or spherical and centric or eccentric in position with a distinct nucleolus. Basophilic (faintly stained) cytoplasm was rich in organelles (see Fig. 3).

1.3. Granulocytes (GRs): spherical to ovoid cells (10-12 µm in diameter) with a centrally located nucleus which might be centric or eccentric (45-55% of the cell volume). The nucleus had a number of scattered chromatin masses and nucleolus. Basophilic (deeply stained) cytoplasm contained a few types of granules, endoplasmic reticulum and an occasional lipid droplet. Some GRs appeared with the extrusion of granules (see Fig. 5).

1.4. Spherulocytes (SPs): basophilic or acidophilic cells (8-20 µm wide, 7-24 µm long), round or ovoid with several cytoplasmic inclusions. They contained either granular, fine-textured filaments or flocculent material. Some cells liberated the entire content of their spherules, leaving on the enclosing membranes. The small, centric, or eccentric nucleus was mostly deformed by the spherules (see Fig. 7).

1.5. Oenocytoids (OEs): the largest circulating hemocytes in the last instar larvae of *G. mellonella*. They are spherical (22-35.5 µm in diameter) or ovoid (18.7-25 µm long, 26.5-35.6 µm wide) cells. After staining with Geimsa stain, the cytoplasm was homogenous basophilic containing clusters of fibrous structures interspersed with very few groups of organelles. The stained small nucleus was slightly eccentric (Fig. 9).

2. Disrupting Effects of PGRs on the Hemocyte Profile:

Depending on the available technique,thelast instar larvae were used because enough haemolymph samples and their cytopathological features could be easily photographed. After continuously force-feeding of 3rd instar larvae on diets supplemented with LC_{50} concentrations of IAA, IBA, 2,4-D and 6-BA, the possible cytopathological impacts on different hemocyte types in haemolymph of last instar larvae could be described as follows.

2.1. Effects of PGRs on Profile of PRs:

In Figure (2), photomicrographs of PRs clearly demonstrated the most important features of deformed cells by the tested PGRs. IAA caused the destruction of the cell membranes and extruded cytoplasmic contents were observed. IBA caused the destruction of the cell nuclei and membranes as well as some extruded cytoplasmic contents were observed. The 2,4-D caused some degeneration of the cell nuclei. The 6-BA caused some degeneration of the cell nuclei and produced some vacuoles in the cytoplasm.

2.2. Effects of PGRs on the profile of PLs:

In Figure (4), photomicrographs of obviously displayed various cytopathological impacts of the tested PGRs on PLs in haemolymph of 7th instar larvae. After continuously force-feeding of 3rd instar larvae on a diet mixed with IBA, degenerated nuclei and vacuolated cytoplasm in PLs could be observed. After force-feeding larvae on a diet mixed with 2,4-D, darkly stained cells with degenerated nuclei had been observed. No cytopathological impacts were seen in PLs after feeding on a diet mixed with IAA or 6-BA.

2.3. Effects of PGRs on the Profile of GRs:

After feeding on a diet mixed with IBA or 6-BA, no cytopathological impacts

were observed in the profile of GRs in haemolymph of the last instar larvae. On the other hand, photomicrographs of Fig. (**6**) indicated some cell deformations, such as vacuolated cytoplasm, after feeding on a diet mixed with IAA or 2,4-D.

2.4. Effects of PGRs on the Profile of SPs:

As shown in the photomicrographs of Fig. (8), some SP deformations, such as degenerative features and vacuolated cytoplasm, could be observed after feeding larvae with a diet mixed with IAA or 2,4-D. In contrast, both IBA and 6-BA failed to affect the morphology and structure of SPs.

2.5. Effects of PGRs on the profile of OEs:

As shown in the photomicrographs of Fig. (9), feeding larvae on a diet mixed with IAA or 2,4-D resulted in some cytopathological features in OEs, such as degenerated cytoplasm. On the other hand, IBA and 6-BA failed to affect the qualitative characteristics of OEs.

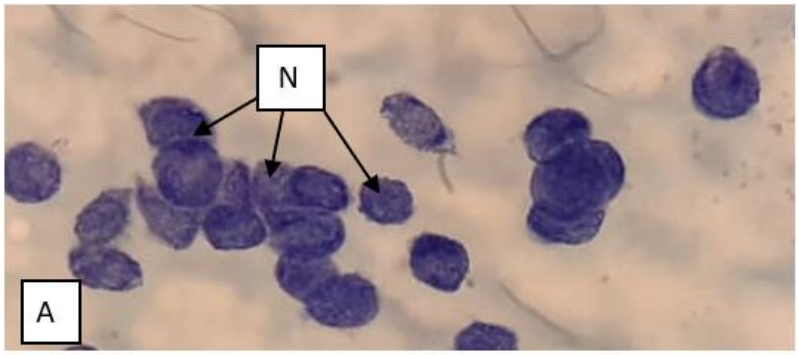

Fig. 1: Photomicrographs of Prohemocytes (PRs) in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A]: Typical normal cells. N: nucleus.

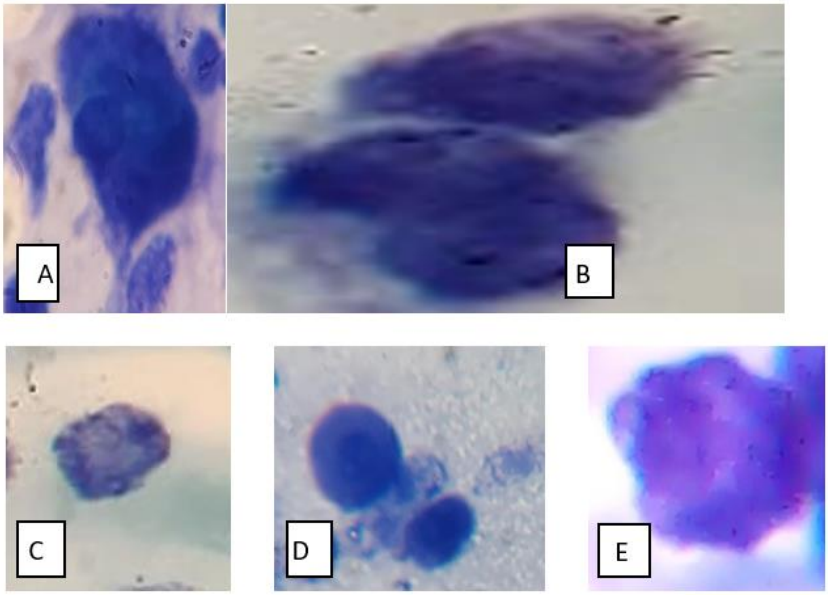

Fig. 2: Photomicrographs of PRs in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A] & [B] darkly stained cells with degenerated nucleus, destroyed membrane and extruded cytoplasmic contents, as deformations caused by 2,4-Dichlorophenoxy acetic acid (2,4-D) and indole-3-acetic acid (IAA), respectively. [E] & [D]: degenerated nuclei, destroyed membranes and extruded cytoplasmic contents. Degenerated nucleus and vacuolated cytoplasm, as deformations caused by indole-3-butyric acid (IBA) and 6-benzyladenine (6-BA), respectively.

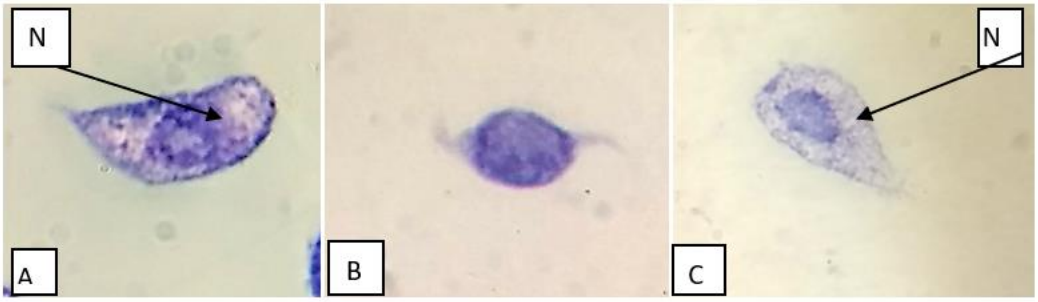

Fig. 3: Photomicrographs of Plasmatocytes (PLs) in the haemolymph of last instar larvae of*G. mellonella* (Geimsa stain, 1000x). [A], [B] & [C]:Typical normal cells. N: nucleus.

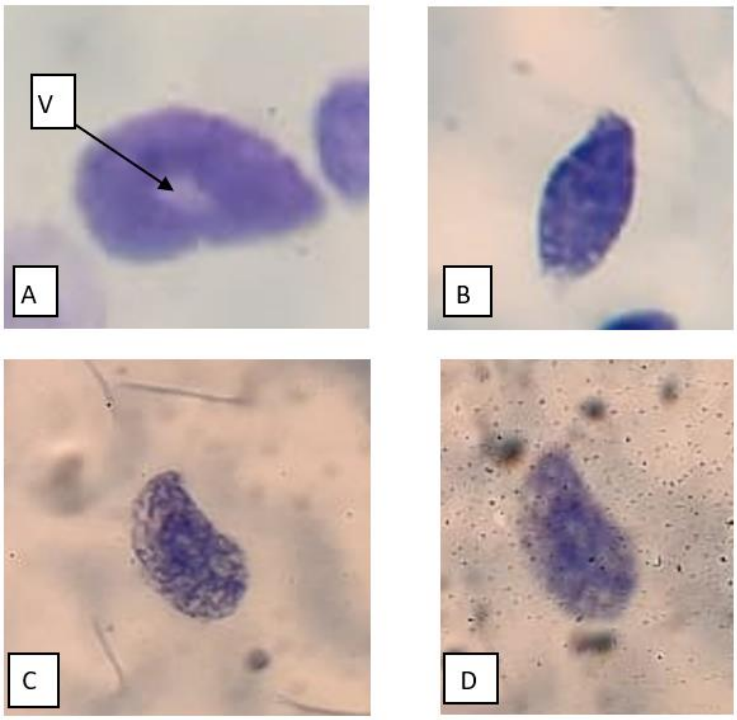

Fig. 4: Photomicrographs of PLs in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A] PLs deformations by IBA: degenerated nuclei and vacuolated cytoplasm. [B] & [C] & [D]: PLs deformations by 2,4-D**:** darkly stained degenerated nuclei. V: vacuole. Both **IAA** and **6-BA** failed to exhibit cytopathological impacts.

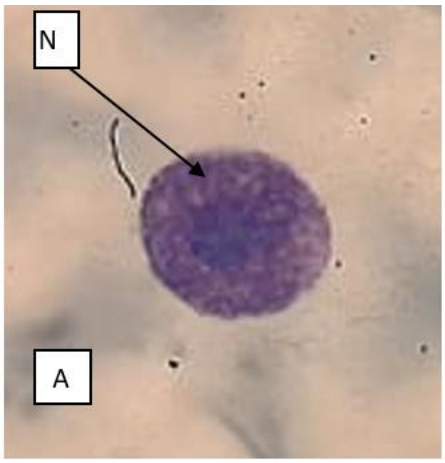

Fig.5: Photomicrographs of Granulocytes (GRs) in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A] Typical normal cells. N: nucleus.

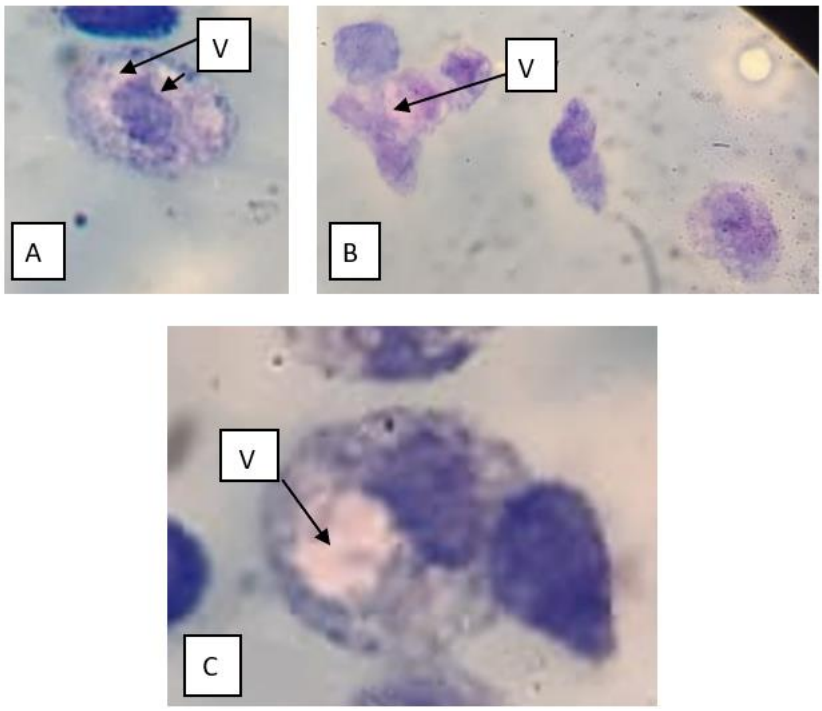

Fig. 6: Photomicrographs of GRs in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). Vacuolated cytoplasm as a feature of GRs deformations, caused by IAA[A] & [B] and 2,4-D[C]. Both IBA and 6-BA failed to exhibit cytopathological impacts.

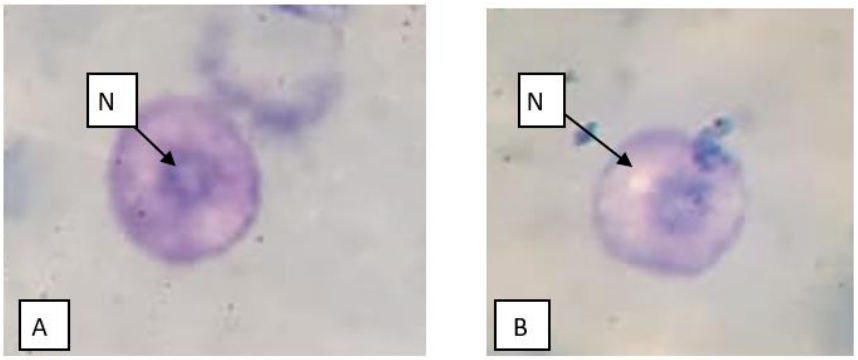

Fig. 7: Photomicrographs of Spherulocytes (SPs) in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A]& [B]:Typical normal cells. N: nucleus.

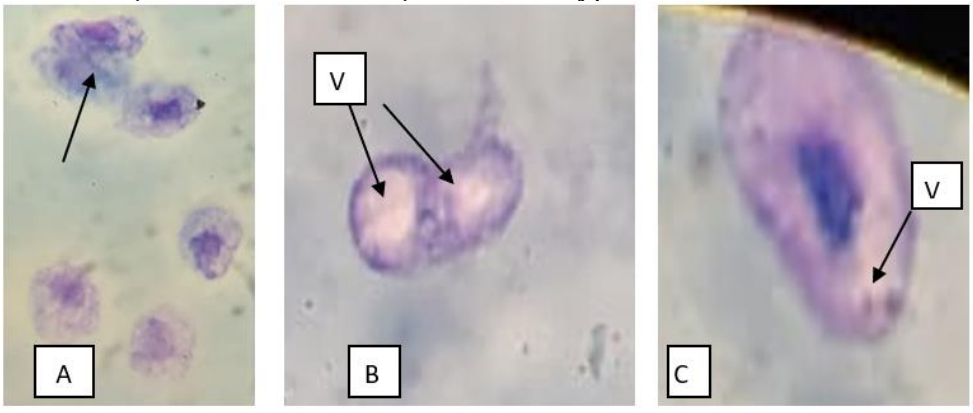

Fig. 8: Photomicrographs of SPs in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A] & [B]: SPs deformations by IAA: degenerated cells. [C], deformations by 2,4-D**:** Oval shaped nucleusand vacuolated cytoplasm. Both IBA and 6-BA failed to exhibit cytopathological impacts.

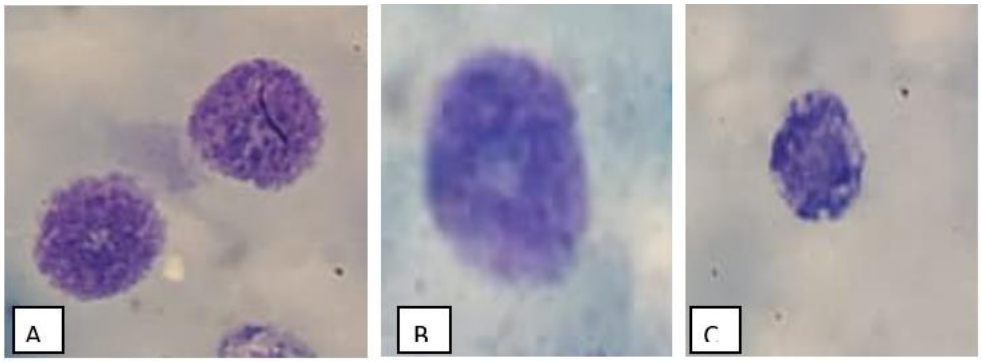

Fig. 9: Photomicrographs of Oenocytoids (OEs) in the haemolymph of last instar larvae of *G. mellonella* (Geimsa stain, 1000x). [A]: Typical normal cells. [B]: OEs deformation by 2,4-D: degenerated cytoplasm [C]: OEs deformation by IAA: degenerated cytoplasm. Both IBA and 6-BA failed to exhibit cytopathological impacts

DISCUSSION

1. Identification of Normal Circulating Hemocytes in Larvae of *G. mellonella*:

Since hemocytes are involved in different insect physiological functions, circulating hemocytes provide an excellent model system to study cell development, differentiation and their role in the immune system (Lavine and Strand, 2002; Rosales, 2011; Pandey and Tiwari, 2012). Therefore, the knowledge of normal hemocytes of an insect is very important to physiologists, toxicologists and biochemists (Qamar and Jamal, 2009; Liu *et al*., 2013). In insects, also, the most common types of hemocytes are prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), adiphohaemocytes (ADs), coagulocytes (CGs) and oenocytoids (OEs). It is important to emphasize that not all of these hemocyte types exist in all insect species (Lavine and Strand, 2002; Meister and Lagueux, 2003; Lamprou *et al*., 2007; Wang *et al*., 2010; Manachini *et al*., 2011) because their diagnostic features are slightly different in various insects (Kanost *et al*., 2004; Ribeiro and Brehelin, 2006; Browne *et al*., 2013). Also, there is confusion between various hemocyte types, such as PRs and PLs as well as GRs and ADs (Nruwirth, 1973). For some detail, see the review of Ghoneim (2019).

In the present study, five main types of the freely circulating hemocytes in haemolymph of the 5th instar and 7th (last) instar larvae of *G. Mellonella* had been identified as PRs, PLs, GRs, SPs and OEs. The identification procedure depended on the cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of the nucleus. The most important diagnostic characteristics of these hemocyte types had been given. This result was in agreement with the reported five hemocyte types in larvae of *G. mellonella* (Altuntaş *et al*., 2012; Kurt and Kayis, 2015; Blanco, 2016; Ghoneim *et al*., 2021). In addition, Sezer and Ozalp (2015) identified five hemocyte types in the pupal haemolymph of *G. mellonella*: PRs, PLs, GRs, SPs and OEs.In contrast, the present result disagreed with many reported results being distinguished by other numbers of circulating hemocytes in *G. mellonella*, such as Shrivastava and Richards (1965) who reported at least three types of hemocytes (PRs, GRs and PLs). Identification of each type by light microscope had often been perplexing, especially for GRs which were difficult to be distinguished from PRs (Ling *et al*., 2003, 2005). Also, three hemocyte types in the haemolymph of *G. mellonella* larvae were observed under a fluorescence microscope (PLs, GRs, and PRs) (Izzetoglu, 2012). In addition, Wu *et al*. (2016) used cytological and morphological analyses for the differentiation of four types of hemocytes in *G. mellonella* (PLs, GRs, SPs and OEs). Er *et al*. (2017) distinguished four types of circulating hemocytes in the last instar larvae of the

same insect (GRs, PLs, PRs and OEs).

To understand the controversial number and types of the circulating hemocytes in *G. mellonella* larvae, it is important to mention that the used nomenclature or terminology for hemocytes has often complicated comparisons of hemocyte categories in different insect orders (Nardi, 2004; Huang *et al*., 2010). For example, the larval hemocytes of Lepidoptera are typically identified by field or phase microscopy whereas this conventional method of hemocyte classification has been the source of frequent controversy in other insect orders (Ling *et al*., 2003). Thus, there is a need to develop a more uniform terminology for naming hemocytes in different insect species (for review, see Ghoneim, 2019). On the other hand, the differencesin the number and types of identified hemocytes in insects maybeattributed to several technical difficulties and the characters adopted by other researchers (George and Ambrose, 2004; Ribeiro and Brehelin, 2006). Various techniques often yield profound different information about the types, number, distribution and functions of hemocytes (for detail, see Ling *et al*., 2005; Qamar and Jamal, 2009; Pandey and Tiwari, 2011; Pandey and Tiwari, 2012). For these reasons, none of the individual methods for studying the various morphological types of hemocytes was entirely satisfactory for all types of cells within a given insect (George, 1996). Therefore, the hemocyte classification has been recommended to be revised several times in the same insect species (Wood and Jacinto, 2007; Siddiqui and Al-Khalifa, 2012 a, b; Ghoneim *et al*., 2015 a).

2. Qualitative Characters (Cytopathology) of the Hemocyte Profile As Disrupted by Plant Growth Regulators (PGRs):

As reportedby many authors, such as El-Kattan **(**1995) for the Indian meal moth *Plodia interpunctella*, Qamar and Jamal **(**2009) for the red cotton stainer *Dysdercus cingulatus*, Teleb**(**2011) for the desert locust *Schistocerca gregaria*, Ghoneim *et al*. (2015 b) for the Egyptian cotton leafworm *Spodoptera littoralis* and Manogem *et al*. (2016) for the lawn armyworm *Spodoptera mauritia*, various insecticides and insect growth regulators exhibited some cytopathological impacts on the circulating hemocytes, basing on the changes in the plasma membrane (erosion and extrusion of their cytoplasmic contents), vacuolization and lysis of the cytoplasm and nuclear disorders. Despite the botanicals represent primarily a source of non-toxic compounds utilized for insect control, different cytopathological effects of botanicals on the hemocytes have been reported only in a few insect species, such as the American cockroach *Periplaneta americana* (Qadri and Narsaiah, 1978), red cotton stainer; *Dysdercus koenigii*(Saxena and Tikku, 1990; Tikku *et al*., 1992), the tobacco cutworm, *Spodoptera litura* (Sharma *et al*., 2003, 2008), the fall armyworm *Spodoptera frugiperda* (Reed and Majumdar, 1998) and *S. littoralis* (Shaurub *et al*., 2014; Waheeb, 2020).

Depending on the current literature, no information was available on the disruptive effects of PGRs on cytomorphology or/and ultrastructure of hemocytes of insects, especially *G. mellonella*. The present study sheds some light on the cytopathological impacts of PGRs, *viz*., IAA, IBA, 2,4-D and 6-BA, on the hemocyte profile of the last instar larvae of *G. mellonella* after a continuously force-feeding of 3rd instar larvae on diets mixed with LC_{50} concentrations of PGRs. Different qualitative disorders of the profile of circulating hemocyte types were recorded.

Disrupted PLs: Treatment of the last instar female nymphs of the grasshopper *Cyrtacanthacristatarica* with Azadirachtin (Azt) led to the bulging of cytoplasm, membrane breakdown and release of cytoplasmic materials from PLs (John and Ananthakrishnan, 1995). Bulging and lysis of PLs were caused by Azt in the last instar larvae of the fly *Psychoda surcoufi* (Ayaad*et al*., 2001). Complete loss of filopods in PLs of the *S. litura* larvae was observed after treatment with Neem gold (an Azt formulation)

(Sharma *et al*., 2003). In contrast, treatment of *S. littoralis* larvae with Azt or its formulation, Margosan-O, could not cause vacuolation in the cytoplasm of PLs (Rizk, 1991). In the present studyon *G. mellonella*, IBA and 2,4-D degenerated the cell nuclei of some PLs in haemolymph of the last instar larvae and produced vacuolated cytoplasm of other PLs. In contrast, IAA and 6-BA failed to exhibit cytopathological impacts on PLs.

Disrupted GRs: Treatment of the last instar female nymphs of *C. tatarica* with Azt resulted in the formation of vacuoles in the cytoplasm and nucleus of GRs (John and Ananthakrishnan, 1995). Also, cytoplasmic projections had been observed in GRs in haemolymph of *S. litura* larvae after treatment with Neem gold (Sharma *et al*., 2003). In the current investigation, IAA and 2,4-D caused vacuolated cytoplasm in GRs of the last instar larvae of *G. mellonella*. In contrast, IBA and 6-BA failed to exhibit cytopathological impacts on the profile of GRs.

Disrupted PRs: The PGRs, IAA and IBA, caused thedestruction of PRs membrane and extruded cytoplasmic contents. The PGRs, 2,4-D and 6-BA, caused some degeneration of the cell nuclei and produced some vacuoles in the cytoplasm.

Disrupted SPs: IAA and 2,4-D caused vacuolated cytoplasm. In contrast, IBA and 6-BA failed to exhibit cytopathological impacts on SPs.

Disrupted OEs: IAA or 2, 4-D caused some cytopathological features in OEs, such as degenerated cytoplasm. In contrast, IBA and 6-BA failed to affect the qualitative characteristics of OEs.

The cytopathological disorders of the identified types of hemocytes in larval haemolymph of *G. mellonella*, in the present study, might be due to the action of some PGRs on the 'actin' which localized in the lamellar extensions of the cells (Anunradha and Annadurai**,** 2008). The question of whether the hemocytes were affected directly or *via* a physiological or endocrinological pathway is yet to be answered.

Conclusion:

Depending on the present study on *G. mellonella*, the PGRs, *viz*., IAA, IBA, 2,4-D and 6-BA exhibited some cytopathological impacts on the profile of the identified five circulating hemocyte types in the last instar larvae, such as destruction of cell nuclei and/or membrane, some extruded cytoplasmic contents, and production of some vacuoles in the cytoplasm. However, some of the tested PGRs failed to exhibit cytopathological impacts on certain hemocytes, since IAA and 6-BA failed to affect PLs, IBA and 6-BA failed to affect GRs, IBA and 6-BA failed to affect SPs and IBA and 6-BA failed to affect OEs. Therefore, IAA and 2,4-D may be recommended to use in the IPM program against *G. mellonella*.

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